

WHAT IS CLAIMED IS:

1 1. A process for preparing a library of DNA fragments of which
2 terminal sequences are known by using a DNA of which base sequence is
3 completely unidentified, which comprises:

4 i) digesting a DNA into fragments which have single-strand
5 cohesive ends by using a restriction enzyme,
6 ii) preparing a series of hairpin loop adapters which have
7 single-strand cohesive ends of which base sequence is known;
8 iii) ligating the DNA fragments with the hairpin loop adapters
9 prepared in the above step ii) by using a DNA ligase; and
10 iv) eliminating the hairpin loop only from the DNA fragments
11 which contain the hairpin loop adapters, obtained in step iii), by using an
12 alkaline solution, an RNase or a single strand specific exonuclease.

13 2. A series of hairpin loop adapters which have single-stand
14 cohesive end, which comprises hairpin loop adapters of which single-
15 stand cohesive ends comprising all sorts of single-strand DNAs which can
16 be obtained by a random combination of four (4) nucleotides.

17 3. A process for selective amplifying DNA of which base
18 sequence is completely unidentified, which comprises:

19 i) digesting a DNA into fragments which have a single-strand
20 cohesive end group by using a restriction enzyme,
21 ii) preparing hairpin loop adaptors which have the single-
22 strand cohesive end which can be complementarily combined to and
23 ligated on the both ends of the DNA fragments obtained in step i);
24 iii) ligating the DNA fragments with the hairpin loop adapters
25 thus prepared by using a DNA ligase;

26 iv) removing DNA fragments and hairpin loop adapters which
27 have not participated in the ligation reaction by using an exonuclease; and
28 v) amplifying the DNA fragments by using a DNA polymerase
29 and a primer which can combine complementarily to a residual sequence
30 from the adapters.

31 4. The process according to Claim 3, which further comprises
32 eliminating hairpin loops from the DNA fragments on which hairpin loop
33 adapter are ligated in step iii).

34 5. The process according to Claim 3, wherein the restriction
35 enzyme is type IIs restriction enzyme.

36 6. The process according to Claim 3, wherein the restriction
37 enzyme is type IIIP restriction enzyme.

38 7. The process according to Claim 3, wherein the DNA ligase in
39 step iii) is T4 DNA ligase.

40 8. The process according to Claim 3, wherein the exonuclease
41 in step iv) is exonuclease III.

42 9. The process according to Claim 4, wherein the hairpin loop is
43 eliminated by using alkaline solution.

44 10. The process according to Claim 4, wherein the hairpin loop is
45 eliminated by using RNase.

46 11. The process according to Claim 4, wherein the hairpin loop is
47 eliminated by using single strand specific exonuclease.

48 12. The process according to Claim 3, wherein the DNA
49 Polymerase is Tag DNA polymerase.